

CELL MECHANICS AND MECHANOBIOLOGY

Hans Van Oosterwyck

Biomechanics section, Department of Mechanical Engineering, KU Leuven, Belgium

Liesbet Geris

Biomechanics Research Unit, U.Liège, Belgium

José Manuel García Aznar

Multiscale in Mechanical and Biological Engineering (M2BE), Aragón Institute of Engineering Research (I3A), Universidad de Zaragoza, Spain

Key Words: mechanobiology, cell mechanics, mechanotransduction, cytoskeleton, computational modelling.

Contents

1. Introduction
 2. Cell mechanics
 - 2.1. Overview
 - 2.2. Experimental techniques to measure cell mechanical properties
 - 2.3. Computational modelling of cell mechanical properties
 3. Cell mechanobiology
 - 3.1. Overview
 - 3.2. Mechanotransduction
 - 3.3. Computational modelling of cell mechanobiology
 4. Conclusions
- Acknowledgements
Glossary
Bibliography
Biographical Sketches

Summary

Mechanical signals are important regulators of cell behaviour. Key to understanding their role is the fact that cells are able to sense and respond to mechanical signals. In order to unravel the interplay between mechanics and biology one needs to embrace experimental and computational methods, stemming from engineering as well as biological disciplines, and integrate them into an interdisciplinary research field called mechanobiology. In this chapter we will first describe the structural and mechanical properties of a cell and its components, as these properties will have important consequences for the way mechanical signals are converted into a biochemical response. Experimental techniques to measure and computational models to capture these properties will be highlighted. Once we have addressed some key aspects of cell mechanics, we will continue by describing some key mechanisms of how mechanical signals can modulate cell behaviour. Again, insights from experimental as well as computational studies will be reviewed. Given the broadness of the field, we will either focus on generic mechanisms, or limit ourselves to a few examples and case studies.

1. Introduction

Mechanical signals are important regulators of organ and tissue development, growth,

remodelling, regeneration and disease. Key to understanding their role is the fact that cells are able to sense and respond to mechanical signals. While the sensory aspect is generally termed mechanosensing, the entire process of sensing mechanical signals and converting them into a biochemical response is called mechanotransduction (see e.g. Ingber (2008) or its definition in the Medical Subject Headings (MeSH®) of the US National Library of Medicine, see <http://www.ncbi.nlm.nih.gov/pubmed/>). The term mechanobiology first appeared in the scientific literature in 1998, and was defined by Dennis Carter as the study of how mechanical or physical conditions regulate biological processes (Carter et al., 1998). At that time, Carter and co-workers were studying the importance of mechanical influences for bone fracture healing. They developed mechanoregulation diagrams that relate local mechanical stimuli to skeletal tissue regeneration, in this way expressing that the local mechanical environment may favour cell differentiation towards specific tissue types. The term mechanobiology was first introduced in a study that did not look at mechanotransduction at the cellular level, but instead made use of well-established engineering methods to calculate mechanical stimuli at the tissue level (such as the finite element method). Similar concepts were also reviewed by van der Meulen and Huiskes in a survey article on (tissue) mechanobiology of skeletal tissues (van der Meulen and Huiskes, 2002). Nowadays, mechanobiology stands for a very interdisciplinary research field that embraces methods – experimental as well as computational - from engineering as well as biological disciplines, among others to unravel mechanotransduction principles. As such, it is not surprising that this book chapter will merge knowledge from both disciplines.

Mechanical loads are present in virtually all organs of the human body in the form of gravitational forces. Organ- or tissue-specific loading conditions can e.g. be found in the musculo-skeletal systems, where muscle loading is responsible for propulsion of the human body, and together with gravitational forces, joint forces and moments determine locomotion. It will lead to the mechanical loading, and therefore the development of local mechanical stresses and deformations of different tissues, such as bone, cartilage, tendon and ligament. Other examples can be found in the cardiovascular system, where the pumping action of the heart is responsible for the development of blood flow and pressure. Cardiac and vascular tissues will be exposed to pulsating hydrostatic pressures and flow induced shear stresses. Other examples are lung tissue, which is cyclically stretched during breathing, and dermal tissues, which are exposed to tensile, compressive and shearing forces. Interestingly, our senses of hearing and touching are also initiated through a mechanical stimulus. In general, the local mechanical environment at the tissue level will be the consequence of the simultaneous action of ‘external’ (i.e. originating from the environment) as well as ‘internal’ (i.e. originating from the tissue itself) forces, leading to tissue deformations that are governed by the ‘passive’ and ‘active’ (contractile) constitutive properties of the cells and tissues respectively (see section 2 for more explanation). As mechanotransduction takes place at the cellular and subcellular level, the study of mechanobiological processes clearly involves a multiscale approach, where mechanical loading needs to be transduced from organ to tissue levels and further down to the cellular and subcellular levels. Similar to the tissue level, we will see that for the mechanical properties of a cell we can make a distinction between a passive and an active component, which will be important for understanding mechanotransduction.

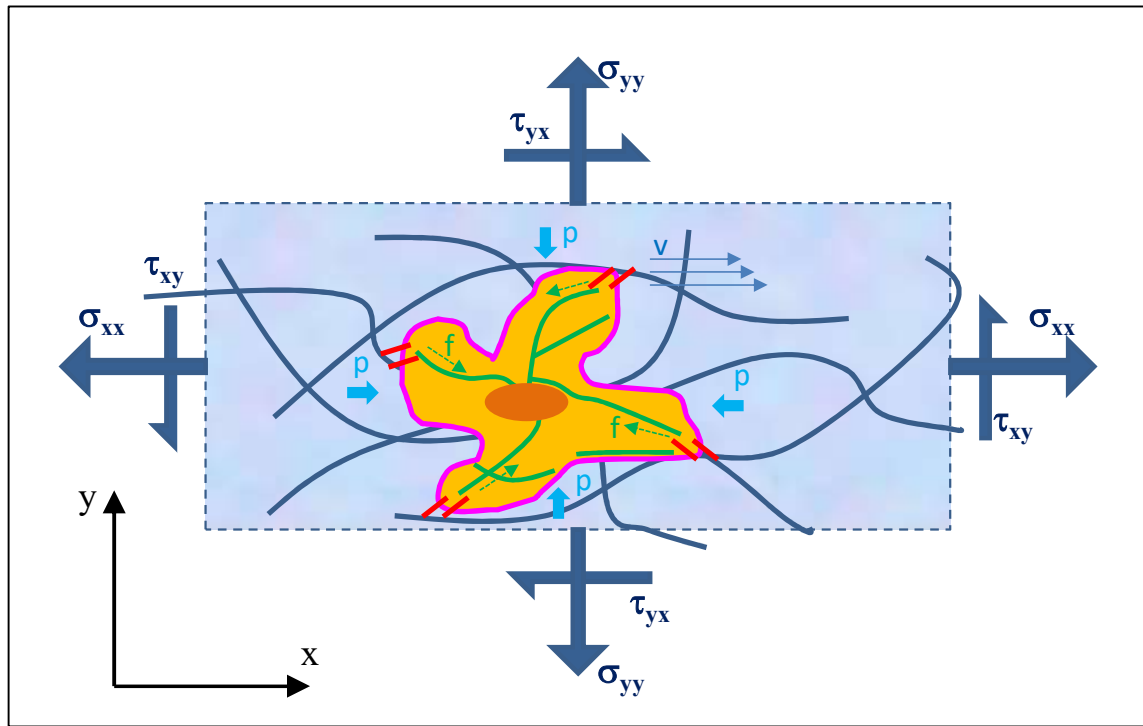


FIGURE 1. Schematic overview of mechanical signals for a cell (cytoplasm = orange, cell membrane= pink, nucleus = brown, cytoskeletal filaments = green) in a hydrated (bluish background), fibrillar extracellular matrix (ECM fibrils = dark blue). Adhesional complexes (red) enable the cell to bind to ECM fibrils. Cytoskeletal filaments are connecting adhesional complexes to the nucleus. External loads lead to matrix stresses (σ = normal stress, τ = shear stress; thick, dark blue arrows) and strains, as well as interstitial fluid pressures (p ; light blue arrows) and fluid velocity fields (v ; thin, dark blue arrows). Tissue deformation can be sensed by the cell through adhesional complexes, which can be further transduced to cytoskeletal filaments (filamentous forces f ; green arrows) down to the nucleus. Fluid velocity fields can lead to drag forces on ECM fibrils as well as cellular components (cell membrane, adhesional complexes), which again may be further transduced via adhesional complexes. Extracellular fluid pressure may be transduced through the cell membrane to the cell's cytosol and organelles. Apart from mechanical signals, induced by external load, the cell's cytoskeletal filaments (through acto-myosin interaction) can exert active, contractile forces (f ; green arrows) on the ECM, which are transduced via adhesional complexes.

A biological system aims at maintaining certain microenvironmental variables at a constant level, a property which is termed homeostasis. In order to do so, the system must possess negative feedback mechanisms that enable to respond to a deviation from the normal (i.e., homeostatic) values of microenvironmental variables, in a way to restore these to the homeostatic values. Well known examples are the regulation of body temperature, blood pH and glucose levels. Interestingly, load-bearing tissues seem to be characterised by homeostasis of mechanical quantities such as stress or strain, meaning that a deviation from a certain 'homeostatic' stress/strain level will induce tissue remodelling or adaptation in order to restore the mechanical integrity (i.e., the respective level of stress/strain). Such a behaviour can be mathematically translated into a simple first order system:

$$\frac{dm}{dt} = C(\psi - \psi_0) \quad (1)$$

where m is a tissue property (e.g. tissue mass, a geometrical or mechanical property), t is time, ψ is a variable accounting for the local mechanical environment, ψ_0 is its value at homeostasis and C is a rate parameter. The concept of stress homeostasis has proven to be very useful for a phenomenological description of tissue remodelling (adaptation) in various tissues, such as the intervertebral disc (Adams and Dolan, 2005), bone (Turner, 1998) or vascular tissues (Humphrey, 2008). As to arterial tissues it is well known from *in vivo* observations that an increase of blood flow (and therefore flow induced shear stress) leads to an arterial enlargement (without wall thickening), while a decreased blood flow (and shear stress) leads to a decrease of lumen diameter (by means of wall thickening at the inner layer, i.e. the intima) (Masuda et al., 1999). Taking wall shear stress as the driving mechanical stimulus and assuming that there exists a homeostatic shear stress value, it becomes clear that for both an increase as well as a decrease of blood flow, the tissue response aims at restoring stress homeostasis, and can therefore be captured by Equation 1. Clearly, this equation does not learn us anything on the cellular mechanisms that underlie this response. *In vitro* experiments are a powerful tool to study these mechanisms, in this case by culturing endothelial cells (the cells that line blood vessels) *in vitro*, and exposing them to controlled regimes of shear stress. Such experiments have clearly demonstrated that endothelial cells are responsive to wall shear stress (for a review see e.g. Ando and Yamamoto (2009)), among others by changing their synthesis of nitric oxide (NO), an important regulator of the activity of smooth muscle cells, which are the cells that are responsible for intimal thickening. The response of endothelial cells to wall shear stress has been found to be a key factor to the growth and destabilisation of arterial plaques in atherosclerosis (Slager et al., 2005). This examples shows the importance of mechanobiology for understanding tissue physiology (arterial remodelling) and pathophysiology (atherosclerosis), and the need for an integrative approach that combines *in vivo*, *in vitro* and *in silico* work. The latter enables to quantify the mechanical environment and to formulate a quantitative relation between this mechanical environment and a biological response (Humphrey, 2008). For an overview of other diseases where aetiology or clinical presentation is associated to abnormalities in mechanotransduction the reader is referred to the review by Ingber (2003).

Prior to describing how mechanical signals can be converted into a biochemical signal, it is a good starting point to first focus on the mechanical properties of a cell and its components, as these properties will have important consequences for mechanotransduction. We will therefore start with a description of the complex structural and mechanical behaviour of the cell, and the way to measure and model different aspects of this behaviour. Once we have established a cell mechanical basis, we will move to cell mechanobiology, present key players of mechanotransduction and examples of computational models of cell mechanobiology. Because of the broadness of the field, it is clearly not possible to give a comprehensive overview. We will therefore either focus on generic mechanisms, or limit ourselves to a few examples and case studies. Often, the reader will be referred to review papers that cover certain aspects into more detail.

2. Cell mechanics

2.1. Overview

An integrated knowledge of cell mechanics is essential for understanding fundamental cellular processes, such as migration, shape stability, proliferation and differentiation. Taken together,

these processes are responsible for the maintenance and regulation of physiological and pathophysiological behaviour of biological tissues. To explain the dynamic and functional role of cells interacting with tissues, it is essential to determine the mechanical properties of cells.

Cell mechanical characteristics are very complex. The cell is a viscoelastic material similar to a viscous fluid (Desprat et al., 2005). Cell viscoelasticity is commonly evaluated through the complex shear modulus $G^* = G' + i G''$, which is defined as the complex ratio in the frequency domain between the applied stress and the resulting strain (Ferry, 1980). The real part (G') is called storage modulus and accounts for the elastic contribution, whereas the imaginary part (G'') is known as loss modulus and represents the dissipative contribution. Dynamic measurements of G^* have revealed that the viscoelastic behaviour of living cells is timescale dependent (Fabry et al., 2001; Stamenovic 2006). Moreover, cell stiffness presents a high variability depending on the conditions and the measurement techniques, varying even several orders of magnitude being, in fact, in the order of tens to thousands of Pascals (Stamenovic and Wang, 2000). Another relevant characteristic of cells that is normally accepted in the literature is that they are a tensed/prestressed structure. In fact, there are filaments that bear a pre-existing tension even in the absence of external loading. Recent results have confirmed that inside the cell there is a filamentous network under tension: when these fibers are cut with a laser, they snap back (Kumar et al., 2006). This internal tension is due to molecular motors that generate forces transmitted by the cell to the extracellular matrix (ECM) (Wang et al., 2001). This internal prestress highly modifies the cell stiffness and its viscoelastic behaviour. Therefore, the cell is characterized by a dual and interactive behaviour: as passive material and active contractile system. The question that still remains unanswered is: which is the factor that regulates the value of this pre-stress within the cell? It seems that the concentration of certain solutes, specifically calcium ions, could control the value of this pre-stress in smooth muscle cells (Stålhand et al., 2008). As will be discussed below, this prestress is also highly important for explaining mechanotransduction phenomena. Another relevant property of cells is their ability to continually rearrange, disassemble and reform its local structures in function of the functionality of the cell in a specific process such as migration, contraction, proliferation and differentiation.

The mechanical properties of the cell are largely determined by four main components with a different contribution: the cytoskeleton (CSK), the membrane, the cytosol and the nucleus. The cytoskeleton (CSK) is a complex, heterogeneous and filamentous structure that extends from the nucleus to the cell membrane providing a continuous and dynamic connection between almost all cellular structures, defining the most significant mechanical characteristics of a cell. In fact, the CSK constitutes the dynamic skeleton of the cell from which the cell is able to change its shape, coordinate its movements, exert mechanical forces and sense the extracellular environment. It consists of a biopolymer network consisting of three major components (see Table 1): filamentous actin (F-actin), intermediate filaments and microtubules. These cytoskeletal polymers are at length-scales (a few microns at most), all corresponding to semiflexible polymers. The thermal fluctuation of one-dimensional semiflexible polymers or filaments is governed by their bending energy and can be characterized using the concept of persistence length L_p . In the absence of thermal fluctuations at zero temperature filaments are straight because of their bending rigidity (K_b). Sufficiently large and thermally fluctuating filaments lose their straight conformation. Only subsystems with contour length $L \ll L_p$ appear rigid and maintain an average straight conformation. Larger filaments $L \gg L_p$, on the other hand, appear flexible. In the 'semiflexible' regime for which L is comparable to L_p , statistical mechanics is governed by

the competition of the thermal energy T and the bending rigidity. In Table 1, we list the persistence lengths and bending rigidities associated to the polymers that constitute the cell CSK (Mofrad and Kamm, 2006).

Table 1: geometrical and mechanical properties of cytoskeletal components

	Diameter (nm)	Persistence length (μm)	Bending stiffness (Nm^2)	Young's modulus (Pa)
Actin filament	6-8	15	7×10^{-26}	$1.3\text{-}2.5 \times 10^9$
Microtubule	25	6000	2.6×10^{-23}	1.9×10^9
Intermediate filament	10	1-3	$4\text{-}12 \times 10^{-27}$	$2\text{-}5 \times 10^6$

F-actin is the main component that regulates the mechanical behaviour of the cell. In fact, its depolymerization implies a significant decrease in cell stiffness (Fabry et al., 2003; Treppe et al., 2005; Smith et al., 2005). In vitro experiments of reconstituted F-actin networks showed that tension sustained by the filaments plays a critical role in the network rheology (Gardel et al., 2006). Actin bundles can bind to myosin, a motor protein able to move the bundles relative to each other by hydrolyzing adenosine triphosphate (ATP), creating what is known as a stress fibre, which is a structure able to support forces in the cell. Therefore, actin filaments in conjunction with myosin are the main force-generating mechanisms of the actin CSK, playing a crucial role in the active behaviour of the cell. Microtubules and intermediate filaments define the main passive behaviour of the cell. Ingber (2003) proposed that cells are prestressed tensegrity structures with internal molecular struts and cables, with microtubules being effective at withstanding compression (the struts), and actin filaments being more adequate for working under tension. He hypothesized this theory based on the fact that microtubules often appear to be curved in living cells, whereas intermediate filaments are almost always linear. This is consistent with the engineering rule that tension straightens and compression buckles or bends the bar elements.

These elements that constitute the CSK create a crowded network of structural proteins that regulates cell shape and drives cell motions, being able to modify and orient this filamentous structure in function of the mechanical and functional needs of the cell in a process known as CSK remodelling (Bursac et al., 2005).

The cell membrane is the layer that separates the cell interior from the extracellular environment. It is formed by a double layer of phospholipid molecules in which proteins are embedded. One of the main functions of the cell membrane is to regulate molecular transport between the cell interior and the extracellular environment. On the other hand, the cell membrane also has a mechanical function, by resisting bending and regulating cell shape.

The cytoplasm is formed by the cell content enclosed within the cell membrane and outside the nucleus. Apart from the CSK, the cytoplasm is formed by the cytosol, which is an aqueous solution formed by a myriad of proteins and molecules that fill the compartments of the cytoplasm.

The nucleus is constituted by two concentrated lipid membranes containing the DNA molecules that encode the genetic information. The CSK biopolymers surround the nucleus in a much higher density than in other cellular regions. In fact, actin and vimentin filaments have been reported to mediate force transfer to the nucleus (Maniotis et al., 1997) with important consequences in gene expression (see also below). The cell nucleus have been reported to be 10-fold stiffer than the surrounding cytoplasm (Maniotis et al., 1997; Gerace and Huber, 2012). Therefore, the nucleus could play an important role in the mechanical stabilization of

the cell (Versaevel et al., 2012)

2.2. Experimental techniques to measure cell mechanical properties

In order to study the complex mechanical behaviour of cells dedicated methodologies have been developed, as described in several review papers (Bao & Suresh 2003; Kasza et al., 2007). One of the complicating aspects is to distinguish between the cell's active and passive behaviour.

In order to evaluate how living cells behave in an active way exerting physical forces, micron-sized probe particles are embedded within the cell or in the surrounding substratum in specific positions to compute the fluctuations in their position as a consequence of cell activity. Particle tracking microscopy (PTM) consists on measuring the motion of probe particles, through video or laser tracking techniques, allowing to study the non-equilibrium phenomena associated to different processes such as thermal fluctuations, the activity of motor proteins, cytoskeletal remodelling, etc. (An et al., 2004; Bursac et al., 2005; Lenormand et al., 2007). Traction Force Microscopy (TFM) techniques (Butler et al., 2002; Sabass, et al., 2008; Legant et al., 2010) are based on the substrate deformation and are used to study the relationship between adherent cells and their underlying substrates.

The passive behaviour of the cell as a material, is strongly non-linear, which is typically found for a soft material, and structurally heterogeneous. This fact requires designing local mechanical experiments with a high accuracy in their measurements. The most common technique used to evaluate the local viscoelastic properties of a single cell is through micro-indentation by Atomic Force Microscopy (AFM) (Sunyer et al., 2009). Since its invention in 1986 (Binnig et al., 1986), it is one of the most valuable tools for imaging and testing matter at the nanometer scale. AFM consists of a microscale cantilever with a tip at its end that allows to apply local stresses to the cell. The cantilever deflection is measured by laser reflection. Alternatively, a local stress can also be applied to a specific region of the cell by twisting or pulling a small magnetic bead that is attached to the cell (or one of its receptors). In Magnetic Tweezers (MT) or magnetic cytometry the resultant bead displacement is measured either with video microscopy or, to an even higher precision, with laser particle tracking. MT have been widely used to measure the viscoelasticity of cells (Bausch et al., 1998). The viscoelastic cell response can also be directly evaluated by deforming the whole cell (Peeters et al., 2005). Recent experiments demonstrate that the elasticity of a whole cell increases dramatically when it is stretched, in agreement with previous tests that related cell elasticity to internally generated prestress (Fabry et al., 2001; Wang et al., 2002; Treppe et al., 2005) and studies of the nonlinear cytoskeletal behaviour (Kollmannsberger and Fabry, 2011). These facts imply that active prestress in the cytoskeleton may be a key parameter that determines cell elasticity. Therefore, it is difficult in the measurements of passive properties of cells to uncouple the effect of active cell properties, because they are continuously present. To evaluate the mechanical properties of the CSK separately, there are *in vitro* studies of reconstituted cytoskeletal networks designed to mimic the properties of individual components of the cytoskeleton (Janmey et al., 2007). A major advantage of these networks is that their viscoelastic properties can be characterized by traditional engineering techniques, evaluating the time-dependent response to an imposed stress or strain.

2.3. Computational modelling of cell mechanical properties

The highly complex mechanical behaviour of the cell makes its modelling very challenging, rendering it currently impossible to achieve a complete model able to take into account all the different known effects under different mechanical conditions. Therefore, as in traditional engineering materials, specific constitutive models have been defined to capture or reproduce specific phenomena of cells under certain mechanical conditions.

Different constitutive models have been presented that describe the mechanics of living cells as a simple elastic, viscoelastic or poro-viscoelastic continuum (Mofrad and Kamm, 2006, Lim et al., 2006), as a porous gel or soft glassy material (Fabry et al., 2001; Bursac et al., 2005; Deng et al., 2006; Mandapu et al., 2008), or as a tensegrity network incorporating discrete structural elements that bear compression (Ingber, 2003, 2008).

Continuum models present several limitations (Mofrad and Kamm, 2006): they normally lack a description of the cytoskeletal fibers and also exclude small Brownian motions caused by thermal fluctuations of the cytoskeleton, which have been shown to play a key role in cell motility (Mogilner and Oster, 1996).

The central hypothesis of the cellular tensegrity model (Stamenovic and Ingber, 2002; Ingber, 2003, 2008; Luo et al., 2008; Moreo et al., 2008) established that some components of the cytoskeletal network are under tension and that these forces are balanced by other cellular components under compression. More recently, several numerical models based on the cellular tensegrity model have focused on the modelling of durotaxis, a process in which cells tend to move from softer to stiffer regions when cultured on a substrate of graded stiffness (Moreo et al., 2008; Lazopoulos and Stamenović, 2008). For variations on the cellular tensegrity model the reader is referred to (Mofrad and Kamm, 2006).

Inspired by the similarity between some experimental data of the mechanical properties of cells and those reported for 'soft glassy materials', specifically those that have probed the dependency of the mechanical properties of cells on the frequency of the applied force, a second model called 'soft glassy rheology' has gained considerable attention (Fabry et al., 2001; Bursac et al., 2005; Deng et al., 2006; Mandadapu et al., 2008). This conceptual model used for soft solids suggests that the cell is composed of an elastic solid with some relaxation process driven by non-thermal stress fluctuations, such as those generated by molecular motors. The predicted mechanical cellular response displays a characteristic timescale dependency that is set by the effective temperature of these fluctuations. This model is rather dependent on the initial probability distribution which is assumed for the system (Mandadapu et al., 2008).

Finally, models based on the physics of semi-flexible polymers provide an excellent description of the behaviour of reconstituted actin polymer networks (MacKintosh, 2006; Gardel et al., 2006; Palmer and Boyce, 2008; Broedersz et al., 2008; Brangwynne et al., 2008). Palmer and Boyce (Palmer and Boyce, 2008) coupled the eight chain Arruda-Boyce network model (Arruda and Boyce, 1993) to an analytical solution of the MacKintosh derivation of the worm-like chain (WLC) model (MacKintosh et al., 1995). Experimental stress-strain curves of reconstituted actin polymer networks (Gardel et al., 2004) could be very well approximated by the model. In contrast to these continuum models, which enable to calculate homogenised network properties, based on an idealised network organisation, computationally much more expensive approaches have been developed by Kim and co-workers (Kim et al., 2009) who use Brownian dynamics to model the interaction between individual actin monomers, filaments and actin binding proteins (ABPs). Such models can represent cytoskeletal networks in an explicit, and therefore much more detailed way, and enable to study the effect of filament rotation and crosslinking stiffness on the network mechanics. More recently, this

discrete approach of the cytoskeleton has been extended in order to incorporate the effect of molecular motors in the actin network, evaluating the capacity of the approach to contract and generate stresses in response to the substrate rigidity (Borau et al, 2012).

Therefore, depending on the research objectives, cells may be modelled quite differently: either as a continuum or as a matrix with fine microstructure; either as a fluid-like or as an elastic material; either as a static structure or as a dynamically evolving system. In any case, a successful model must incorporate the recent experimental evidences highlighting the importance of nonlinear mechanics and internal cellular pre-stress.

3. Cell mechanobiology

3.1. Overview

As mentioned in the introduction, external mechanical loading that results from (patho)physiological activity is a potent regulator of in vivo cell and tissue function. A vast amount of in vitro studies can be found in the literature that have applied controlled external loading to cell culture systems, in order to investigate the cellular response to mechanical stimuli. Measurement of the cellular response can be very diverse, and depending on the application encompasses the activation of intracellular signalling cascades (short term responses, such as phosphorylation reactions), gene expression (mRNA synthesis), protein secretion (such as extracellular matrix proteins), change in cell shape (cytoskeletal reorganisation) and more long term biological responses such as cell survival (apoptosis), proliferation, differentiation and matrix remodelling (as for tissue engineering applications, see below). Depending on the physiological conditions that one likes to reproduce, in vitro setups have been used that enables to apply (static or dynamic) uniaxial stretching, biaxial stretching, hydrostatic pressure and fluid flow induced shear stress to cells grown in monolayer (i.e. cells grown on a flat substrate) (see Orr et al. (2006) and Wang and Thampatty (2006) for review papers across multiple tissues and cell types). Techniques for applying very localised mechanical stimuli (such as by means of magnetic bead cytometry) have been applied not only for measuring cell mechanical properties, but also to study their effect on intracellular biochemical reactions that are part of the mechanotransduction cascade (Wang et al., 2009).

Mechanical stimulation also forms an important part of many in vitro tissue engineering strategies that aim at creating viable, (mechanically) functional tissues or tissue intermediates that once implanted in vivo, can integrate with the host tissue and restore tissue function (Butler et al., 2009). The term functional tissue engineering has been coined to emphasise on the importance of mechanical properties and mechanical stimulation for the development of successful tissue engineering products (Butler et al., 2000). As native tissues require a multicellular, three-dimensional (3D) organisation that is supported by an extracellular matrix, a key aspect to mechanobiological studies in tissue engineering is the application of external mechanical loading to cellular constructs, in which cells are cultured in a 3D environment. Depending on the application, the 3D environment can be created by using macroporous scaffolds, micro- or nanoporous hydrogel carriers or without the use of any carrier system. In the latter case, the self-aggregating properties of cells can be employed to create a 3D, multicellular environment, in which cells create their own ECM. Again, depending on the in vivo mechanical conditions that one likes to recreate in a bioreactor environment, mechanical loading can be composed of construct deformation – compressive, such as for cartilage or bone engineering; tensile, such as for tendon, ligament or muscle engineering -, hydrostatic pressure – such as for cartilage engineering -, or fluid flow –such as

for cardiovascular or bone applications. More complex systems that combine different loading modes (e.g. pulsatile pressure and flow for vascular tissue engineering, compression and shear for articular cartilage engineering – have been developed as well. As many bioreactor systems – such as perfusion bioreactors, stirred tanks, rotating vessels - make use of advection to enhance mass transport –either around or through the cellular construct – flow induced shear stresses will contribute to the mechanical environment. As cells are now residing into a much more complex 3D environment (that may be heterogenous and time-dependent due to matrix remodelling), the quantification of the mechanical stress, strain and/or flow states requires the use of much more advanced techniques that can be either experimental, computational, or both. The development of appropriate constitutive models of the cellular constructs –either at the tissue scale to capture the macroscopic behaviour, or multiscale to relate tissue and cell mechanical behaviour – forms an important and integral part of a computational effort. The role of mechanical loading for the engineering of specific tissues has been reviewed in a number of papers (Darling and Athanasiou, 2003; Nerem, 2003; Benhardt and Cosgriff-Hernandez, 2009; Butler et al., 2009).

ECM mechanical properties

Apart from external loading, the mechanical properties of the cells and their extracellular matrix strongly contribute to the local micromechanical environment. As mentioned earlier, thanks to its active, contractile properties a cell can apply traction forces to its extracellular environment (the extracellular matrix, but also to neighbouring cells). The net extracellular and intracellular stress and strain state will therefore be a function of external loading, ECM mechanical properties and cell mechanical properties (passive as well as active) (see also below, in particular the discussion on the work of Moreo et al. (2008)). Many in vitro experiments have demonstrated the importance of the ECM / substrate stiffness for cellular responses, such as cell spreading (cell morphology), adhesion, migration, apoptosis, proliferation and differentiation (Georges and Janmey, 2005; Chen, 2008; Zajac and Discher, 2008). These findings allude to the key role of ECM mechanics for many physiological and pathophysiological processes during development, regeneration and disease, such as the determination of stem cell fate (Guilak et al., 2009), the formation of blood vessels (vasculogenesis and angiogenesis) (Ingber, 2002) and tumour metastasis (Kumar and Weaver, 2009).

In the context of tissue regeneration and engineering, many recent studies have focused on the importance of the microenvironment for modulating stem cell lineage specification. The in vivo microenvironment that stem cells are exposed to – the so called stem cell niche – is likely to be tissue-specific, as native tissue properties can be very diverse. With the exception of mineralised bone and tooth, tissues are soft, with elastic moduli ranging e.g. from 0.1-1 kPa for brain tissue, 10 kPa for muscle tissue and 20-40 kPa for non-mineralised bone (Zajac and Discher, 2008). Although fully mineralised bone tissue is very stiff (Young's moduli of the order of 10-30 GPa, (Zysset, 2009)), it is originally deposited as a non-mineralised, highly compliant collagenous phase (so called osteoid). To some extent, the mechanical microenvironment can be recreated in vitro, by culturing stem cells on substrates with a 'tissue-specific' elasticity. Various hydrogel systems exist that allow to modulate the elastic properties without substantially changing their chemical properties (Lutolf et al., 2009). Polyacrylamide gel systems have been used as cell culture substrates, as they enable to control the substrate Young's modulus over three orders of magnitude (0.1-10 kPa) (Zajac and Discher, 2008) by varying the degree of cross-linking. At the same time, cell adhesion can be controlled by covalently attaching collagen I. As the stiffness (e.g. force per unit of

displacement) is a function of intrinsic elastic gel properties as well as geometrical properties, the gel thickness needs to be controlled as well.

Engler et al. (Engler et al., 2006) cultured mesenchymal stem cells on collagen I-coated polyacrylamide gels with brain-like (0.1-1 kPa), muscle-like (8-17 kPa) and non-mineralised bone-like (25-40 kPa) Young's moduli and demonstrated that cell morphological changes and stem cell commitment to a certain phenotype were strongly regulated by substrate elasticity. Interestingly, phenotypic changes matched the elastic properties of the native tissues, i.e. differentiation (as assessed by the expression of cell-specific marker genes, such as transcription factors, and morphological changes, such as cell branching) to neuron-like, myoblast-like and osteoblast-like behaviour was enhanced on brain-like, muscle-like and bone-like substrate elasticities respectively. These phenotypic changes could be induced in normal growth media, in the absence of any specific chemical (soluble) factors that are known to induce differentiation. When such factors were added to the culture medium, a synergistic effect could be noticed between the chemical and mechanical environment, with higher expression levels in induction media that still peaked for the same tissue-specific substrate elasticity. When chemical factors were added to the medium that interfered with the cell's contractility (addition of blebbistatin, a nonmuscle myosin II inhibitor), neither phenotypic changes nor any effect of substrate elasticity could be seen.

These findings could be confirmed for mature, differentiated cells, such as mature myoblasts and osteoblasts, for which the highest expression levels were reported when these cells were cultured on 'myogenic' and 'osteogenic' substrate elasticities respectively. These results clearly demonstrate the importance of and interplay between the mechanical and biochemical microenvironment for controlling stem cell fate. While the work of Discher and co-workers considered cells on flat substrates, the work of Huebsch and co-workers have confirmed the importance of ECM stiffness for in vitro stem cell commitment towards osteogenesis, when culturing mesenchymal stem cells in a 3D hydrogel environment (Huebsch et al., 2010). Other studies showed that the elasticity of the substrate used to culture stem cells in vitro can also affect their regenerative capacity when implanting these cells in vivo. Gilbert and co-workers demonstrated that only when muscle stem cells were cultured on substrates with elasticity resembling that of muscle tissue, they contributed extensively to muscle regeneration in vivo (Gilbert et al., 2010).

3.2. Mechanotransduction

While we have previously described the importance of cellular tensegrity for cell mechanical behaviour, it is also a key element in mechanotransduction theories. The transduction of a mechanical signal from the extracellular to the intracellular space (and vice versa) as well as the mechanochemical transduction of a mechanical signal into a biochemical response can be seen as distinct, although related aspects of mechanotransduction. Mechanical signals that originate at the level of the extracellular matrix (e.g. as a consequence of external, physiological loading) can be transduced to the nucleus through a molecular network that connects the ECM to the nucleus (Wang et al., 2009). Cells are anchored to the ECM by means of integrins, a specialised class of transmembrane proteins. Their extracellular domain enables them to bind to the ECM (or substrate). Integrin molecules are heterodimeric transmembrane receptors and composed of an alpha and beta subunit, the combination of which results in a binding affinity which is specific to certain ECM proteins (Huvener and Danen, 2009). Their cytoplasmic domain binds to actin-associated proteins, such as talin, vinculin, zyxin and paxillin (Wang et al., 2009). Certain integrin proteins (e.g. $\alpha\beta4$) can also

bind to intermediate filaments. Integrins cluster into specialised anchoring complexes, called focal adhesion complexes (FACs) which are essential to transmit mechanical signals across the cell membrane from the ECM to the cytoskeleton. Apart from their importance for force channelling, the cytoskeletal backbone of the FACs also associates to multiple signalling molecules (Ingber, 2008). While integrins constitute molecular bridges between the ECM and the cytoskeleton, cadherins, which mediate cell-to-cell adhesion, form junctional complexes as well, which contain β -catenin and γ -catenin that bind actin filaments and intermediate filaments respectively (Wang et al., 2009).

Apart from molecules that link integrins to actin, other molecular players have recently been identified that together provide a molecular connectivity from the ECM through integrins and the cytoskeleton to the nucleus. For further information the reader is referred to the review of Wang et al. (Wang et al., 2009). This interconnected molecular framework provides a means of channeling mechanical signals from the ECM throughout the cell. Pioneering work has been done by Ingber and co-workers, which has been summarized in many review papers, and which has demonstrated the importance of tensegrity for cellular mechanotransduction (see e.g. (Ingber, 1997; Ingber, 2006; Ingber, 2008)). Their work has provided evidence that cells do not sense mechanical signals through membrane deformation, but through cell surface adhesion receptors that couple the cytoskeleton to the ECM. Ingber and co-workers showed this by means of a magnetic cytometry technique, in which a controlled mechanical load was applied to magnetic microbeads that were attached to various membrane receptors. It was found that cell stiffening (increase of stiffness with increase of load application) only occurred when magnetic beads were attached to integrins, and not when attached to receptors that do not form focal adhesions (such as growth factor receptors) (Wang et al., 1993; Yoshida et al., 1996). More recently, an intracellular traction force microscopy technique has been developed that enables to calculate stress distributions in the cytoplasm of the cell. Small mechanical deformations applied to surface integrins (by means of magnetic cytometry) resulted in long range force propagation, where stress concentrations could be noticed in locations far away from the site of force application, including close to the nucleus and at the opposite side of the cell (Hu et al., 2003). In addition, long range force propagation did not occur when the cytoskeleton was disrupted or when cytoskeletal prestress was inhibited. The latter finding demonstrates the importance of having a 'hard-wired' tensegrity structure for an efficient propagation of mechanical signals throughout the cell. These experiments also demonstrate the validity of the long range structural rearrangements predicted by computer models of cellular tensegrity. In addition, other in vitro experiments have demonstrated that such locally applied mechanical signals can lead to rapid protein activation (within 0.3 s) at remote cytoplasmic sites, while activation of the same protein by means of soluble growth factors only took place after 12 s of stimulation (Na et al., 2008).

The fact that an interconnected, pre-stressed ('hard-wired') molecular framework supports the cell is not only important for force propagation, it also provides mechanisms for the conversion of a mechanical signal into a biochemical response (the second aspect of mechanotransduction). This among others has to do with the fact that many enzymes and substrates that govern DNA synthesis, transcription, RNA processing, protein synthesis, glycolysis and signal transduction are immobilised on the cytoskeleton, which serves as a scaffold for biochemical reactions (Ingber, 1997). Mechanical signals that are focused on the cytoskeleton may lead to deformation (conformational changes) of the molecules that are associated to it, which may alter their biochemical activity. Given the fact that focal adhesion complexes are 'integrators' of many signalling molecules, they may be key sites of mechanochemical signal transduction. Because mechanical signals can propagate from the

ECM to the nucleus, there may be many candidate mechanosensory proteins which undergo a conformational change, such as an ECM protein, a transmembrane protein, an intracellular protein or a molecule found in the nucleus (Janmey and Weitz, 2004). Wang et al. proposed different mechanisms for mechanochemical conversion in the nucleus (Wang et al., 2009). Forces propagated to nuclear membrane-spanning receptors may lead to changes in chromatin and/or nuclear scaffold organization, in turn affecting gene regulation (e.g. by means of transcriptional regulation). They may also influence the opening and closing of nuclear pores, thereby modulating mRNA transport (post-transcriptional regulation). Finally, forces may lead to stretching of specific regions of the DNA (through matrix attachment regions), in this way promoting DNA melting and binding of transcriptional regulators.

In their review paper Wang & Thampatty (Wang and Thampatty, 2006) discern a number of cellular components that are important in the mechanochemical conversion that underlies mechanotransduction. Apart from integrins and the cytoskeleton, they mention G proteins, receptor tyrosine kinases (RTKs), mitogen-activated protein kinases (MAPKs) and stretch-activated ion channels:

G proteins (GTP binding proteins) are a family of plasma membrane proteins that strongly bind guanine nucleotides. G proteins need to be activated by an other membrane-bound receptor (G-protein coupled receptor), which in the case of chemical activation, in turn will be activated by binding an extracellular chemical signal (the so called ‘first messenger’). In its inactive form G proteins bind guanosine diphosphate (GDP), while upon activation by an activated receptor GDP is released and instead they bind guanosine triphosphate (GTP). The active G protein can then interact with effector proteins in the membrane (which can be enzymes or ion channels) in order to modulate their activity. Activation of these effector proteins can lead to the generation of so called ‘second messengers’, like cyclic-AMP (cAMP), diacylglycerol (DAG), inositol triphosphate (IP3) and calcium ions. Further downstream this can regulate metabolic enzymes, ion channels, transporters, and other components of the cellular machinery, ultimately controlling a broad range of cellular processes, including transcription, motility, contractility, and secretion (Neves et al., 2002). Activated G proteins have GTPase activity, meaning that they will remove a phosphate group from itself, returning to their inactive state.

As to their importance for mechanotransduction, G protein subunits have been found to be localized at focal adhesion sites (Hansen et al., 1994). In vitro studies have demonstrated G protein activation in different cell types as a result of mechanical loading, such as in the case of uniaxial stretching (Clark et al., 2002) and fluid flow induced shearing (Gudi et al., 1996) of endothelial cells, or equibiaxial stretching of cardiac fibroblasts (Gudi et al., 1998). Meyer et al. (Meyer et al., 2000) showed that G-protein dependent cAMP signaling was induced by mechanically shearing integrin receptors (through magnetic twisting cytometry), while the application of the same load levels to transmembrane metabolic receptors did not cause any effect.

Receptor tyrosine kinases (RTKs) are a family (consisting of 20 subfamilies of protein kinases, i.e. enzymes that phosphorylate other proteins by transferring a phosphate group from ATP to the protein (Zwick et al., 2001). These kinases phosphorylate the tyrosine residues of the target protein (other proteins as well as the receptor itself, i.e. receptor autophosphorylation). In the case of RTK the enzyme is itself a transmembrane receptor that

upon binding an extracellular first messenger (again in the case of 'traditional' chemical activation) gets activated, meaning that the conformation of its intracellular domain (which is related to its enzymatic activity) is changed. Targets of the activated RTK can be either enzymes, such as Src and phospholipase C γ , or adaptor molecules that link RTK activation to downstream signalling pathways, such as mitogen-activated protein kinase signaling (see below). RTKs have been shown to be key regulators of normal cellular processes but also to play a critical role in the development and progression of cancer. Many growth factor receptors belong to the RTK family, such as the Epidermal growth factor receptor (EGFR), Fibroblast growth factor receptor (FGFR), Vascular endothelial growth factor receptor (VEGFR), Insulin growth factor receptor (IGFR) and Platelet-derived growth factor receptor (PDGFR) family. Some of these growth factor receptors can associate to the cytoskeletal backbone of the focal adhesion complex, suggesting that the focal adhesion complex can act as an integrator of integrin and growth factor signaling (Plopper et al., 1995; Ingber, 2008). Crosstalk between integrin and growth factor signaling has been demonstrated, e.g. as reviewed in the context of cancer cell growth, survival, and invasion (Soung et al., 2010), as well as of endothelial cell behaviour during angiogenesis (Eliceiri, 2001). Eliceiri states that integrin ligation is required for growth factor-induced biological processes, and that integrins can directly associate with growth factor receptors, thereby regulating the capacity of integrin/growth factor receptor complexes to propagate downstream signaling (Eliceiri, 2001).

Mitogen-activated protein kinases (MAPKs) are an important group of signal transducing enzymes that phosphorylate serine or threonine residues on proteins, upon activation by means of an extracellular signal, such as a mitogen (i.e. a chemical factor that enhances mitosis) (Chang and Karin, 2001). MAPK signaling cascades are important for many aspects of cellular regulation, such as gene expression, cell proliferation and apoptosis (i.e. programmed cell death). These cascades involve three subsequent modules of phosphorylation, starting with the activation of a MAPKK kinase or MEK kinase (MAPKKK or MEKK), which upon activation by means of an extracellular signal phosphorylates a MAPK kinase (MAPKK, MKK or MEK), in turn phosphorylating a MAPK. In mammalian cells at least four distinct groups of MAPKs have been discovered: extracellular signal-related kinases (ERK1/2), Jun amino-terminal kinases (JNK1/2/3), p38 proteins (p38 $\alpha/\beta/\gamma/\delta$) and ERK5. Each group is activated by specific MAPKKs, while each MAPKK can be activated by more than one MAPKKK. MAPK signaling cascades can be activated by G-protein coupled receptors (Chang and Karin, 2001) as well as RTKs (Rossomando et al., 1989; Zwick et al., 2001), demonstrating potential crosstalk between those signaling cascades.

As reviewed by Wang and Thampatty (Wang and Thampatty, 2006), mechanical loading has been demonstrated to activate (phosphorylate) ERK1/2, JNK and/or p38 in many different cell types, such as aortic endothelial cells (when exposed to shear stress), pulmonary epithelial cells (in response to stretching), articular chondrocytes (compression), cardiac fibroblasts (stretching) and myocytes (stretching). Some of these MAPKs may be physically associated to the cytoskeletal backbone of the focal adhesion complex, rendering it again the primary site for mechanotransduction (Ingber, 2008).

Stretch-activated ion channels

Ion channels can be activated by means of mechanics, providing another mechanism for mechanotransduction (Hamill and Martinac, 2001). Their opening and closing dynamics regulates the intracellular ion concentration, which in turn will affect downstream molecular events. Owing to the regulation of a wide range of processes by means of intracellular

calcium, stretch-activated calcium channels have been studied extensively. As reviewed by Wang and Thampatty (Wang and Thampatty, 2006), mechanical stimulation affects the concentration of intracellular calcium in many cell types, including smooth muscle cells, fibroblasts, osteoblasts and endothelial cells. They may control the mechanically-induced activation of other signal transducing molecules, such as RTKs and MAPKs. This was e.g. shown by (Iwasaki et al., 2000) for the mechanical response of vascular smooth muscle cells. Cyclic stretch was found to induce a rapid activation (phosphorylation) of EGFR (within 2 min) and ERK1/2 (within 5 min). The phosphorylation of ERK1/2 was inhibited by addition of an inhibitor of EGFR kinase activity, demonstrating that EGFR was upstream of ERK1/2. When cells were treated with a stretch-activated calcium channel blocker stretch-induced phosphorylation of EGFR and ERK1/2 was inhibited. These data suggest that stretch-activated ion channels can be crucial for mechanically induced EGFR and ERK1/2 activation.

In recent years, many molecular players have been identified that are involved in mechanotransduction through integrin activation. The crosstalk between integrins, Src-family kinases (SFKs) and Rho-family GTPases seems to be key to the way physical signals from the extracellular matrix can regulate cellular processes that are important for normal tissue function and disease (see Huveneers and Danen (2009) for an extensive review). Upon activation integrin promotes the autophosphorylation of focal adhesion kinase (FAK), which leads to binding to Src, in turn leading to the phosphorylation of other tyrosine residues on FAK, thereby maximizing its kinase activity and creating additional protein binding sites (Mittra and Schlaepfer, 2006). Src belongs to a family of non-receptor tyrosine kinases, its name being short for 'sarcoma', as it was originally discovered as a proto-oncogene (i.e. a normal gene that upon mutation can become an oncogene and therefore cause cancer) (Stehelin et al., 1977; Oppermann et al., 1979). Both Src and FAK, also a non-receptor tyrosine kinase, are found to be associated to the cytoskeletal backbone of focal adhesion complexes.

The active FAK-Src complex acts on guanine-exchange factors (GEFs) and GTPase-activating proteins (GAPs) that together control the activity of a number of Rho-GTPases. These are a subfamily within the Ras superfamily of small GTPases (small, because these GTP-GDP binding enzymes contain only one unit, in contrast to the previously mentioned G proteins that are composed of three subunits), which together control the growth and contraction of filamentous actin (F-actin), through proteins such as actin-related protein 2/3 (Arp2/3) and myosin. The Rho-GTPases are further subdivided into RhoA, Rac1 and Cdc42. Each of these GTPases has its own GEFs (that activate the GTPase) and GAPs (that inactivate the GTPase) that together regulate its activity.

RhoA activation will lead to stress fiber formation and enhanced actomyosin contractility (through activation of one of RhoA's effector proteins, namely ROCK). The activation of Rac1 and Cdc42 are both associated with cell spreading, i.e. the formation of membrane protrusions, such as lamellipodia (Rac1) and filopodia (Cdc42). Interestingly, RhoA and Rac1 suppress each other's activity, which could be important for coordinating cytoskeletal (F-actin) reorganisations during different stages of cell spreading. This balance between RhoA and Rac1 is also essential for directional cell migration, which relies on tightly regulated spatiotemporal changes of cytoskeletal structures. This balance will be different in the retracting rear and the leading edge of the cell body, which is mediated by the expression of different integrins. In the retracting rear, RhoA activity will be high, so that Rac1 activity will be inhibited, leading to enhanced actomyosin contractility and inhibition of the formation of

membrane protrusions. In contrast, enhanced Rac1 activity in the leading edge will lead to the formation of the lamellipodium, at the same time inhibiting RhoA induced contractility.

As mentioned earlier, integrin and growth factor signaling can interact with each other (Eliceiri, 2001; Soung et al., 2010). SFKs and Rho-GTPases provide several mechanisms for crosstalk, such as the direct interaction of autophosphorylated growth factor receptors with either SFKs (Bromann et al., 2004) or GEFs or GAPs (Burridge and Wennerberg, 2004). In addition, it has been shown that growth factor receptor activation (phosphorylation) can be directly induced by integrin-mediated Src activation. This was e.g. demonstrated for epidermal growth factor receptor (EGFR), which could be phosphorylated in the absence of any extracellular EGF (Moro et al., 2002).

3.3. Computational modelling of cell mechanobiology

As we have shown in the previous section, there are multiple evidences that point at the relevance of mechanics in cellular processes such as migration, proliferation and differentiation. The way cells sense and respond to their mechanical 3D environment is complex and dynamic, and results from the integrated effect of the mechanical properties of the extracellular matrix (ECM) and cell cytoskeleton (CSK), the biology of the ECM, the cell mechanotransduction properties and the transport of secreted molecules whose local gradients affect the cell response. Therefore, one of the challenges that researchers nowadays face is to understand how physical forces and mechanical structures contribute to genetic and cellular regulation. In recent years, it has become increasingly popular and important to use computational models to understand these mechanobiological mechanisms. Different modelling methodologies have been used, although they could be mainly divided into three categories according to the spatial scale they are based on: molecular, cellular and tissue scale (Grima, 2008). Most models approach mechanobiology at the tissue scale, few address the cellular scale and very few address the molecular scale. In this section a brief revision of cellular models is presented, because these models allow to address the importance of cell-ECM and cell-cell interaction for cell proliferation, migration and differentiation.

Macroscopic or tissue level models are very useful for making qualitative predictions, but may fail in spatial regions where cell concentration is small (and therefore where a continuous description is not valid anymore), and they do not account for a mechanistic treatment of mechanobiological processes. Models at the cell scale are the most common strategy, where the cell mechanical behaviour is simulated by the different constitutive laws shown in subsection 2.3. Recently, a strong focus has been established to create cell-specific Finite Element models for simulating realistic 3D cellular geometries from confocal microscopy images (Slomka and Gefen, 2010; Or-Tzadikario and Gefen, 2011; Wood et al, 2012). Finally, molecular models are attractive for studying mechanotransduction inside a cell. These kinds of models are starting to be developed to understand the cell mechanical behaviour under different conditions (Mofrad and Kamm, 2009).

One of the process more widely simulated in biology is **cell migration** and how mechanical conditions regulate this migration. Therefore, in this section, some computer models to simulate these phenomena from continuum to discrete approaches will be presented.

The classical approach to model collective cell movement is through coupled non-linear reaction-diffusion equations for cells and diffusive chemicals that interact with the cells, without taking into account the role of mechanics (Murray, 2002). Some first approaches have been proposed from a phenomenological point of view, considering the effect of mechanics

indirectly, but not based on a real mechanistic approach (Moreo et al, 2008; Geris et al., 2010). However, these continuum approaches have allowed the analysis of multiple complex phenomena of morphogenesis (Murray, 2003; Moreo et al, 2010), wound healing (Javierre et al., 2009; Valero et al., 2012), bone healing (García-Aznar et al, 2005; Geris et al., 2009), bone formation around implants (Amor et al., 2009; Prokharau et al., 2012) and other applications.

Another different approach that has been used to simulate these processes considering cell-cell and cell-matrix interactions are multicellular models. These models take into account biochemical kinetics at a subcellular scale, but these models present limited impact on the interaction with the local environment, especially with the mechanical conditions of the environment and the cell mechanical properties. There are many ways of modelling morphogenetic development in multicellular approaches. Some of the most common approaches are briefly described here. **Fluid type models** explore cell sorting where the cell aggregate is considered a mixture of two fluids (Umeda and Inouye, 2004). The main limitation of these models is that it is very difficult to take into account effects like cell size and stiffness and active force generation by the cells. Models using a **cellular automata** or **cellular Potts** approach have been widely used in morphogenesis modelling (Merks et al, 2009) and have been incorporated in software, such as CompuCell3D (Chaturvedi et al, 2005). This software has been designed to model 3D morphogenesis processes, and combines the Cellular Potts model with a continuum reaction-diffusion model and a state automaton to consider cell differentiation transitions. Nevertheless, this model also present important limitations, in fact, it does not incorporate mechanical factors of cell-cell and cell-matrix interactions. More recently, different approaches have been presented to model collective cell migration. Jamali et al (2010) simulated a growing epithelial cell culture in 2D by means of different subcellular viscoelastic elements. Vermolen and Gefen (2012a, 2012b, 2013) also developed different discrete approaches for modelling continuous movement of cells incorporating cell changes during migration. Rey and García-Aznar (2013) also simulated collective cell movement analysing the different behaviour of the colony in function of the relation between the cell propulsion force with the substrate and the cell-cell force interaction. In any case, all these approaches are mainly focused on the simulation of 2D collective cell migration.

However, the number of mathematical models simulating cell motility in a 3D extracellular matrix is very small (Rangarajan and Zaman, 2008). These can be divided into two categories: those models that predict individual cell motility and others that predict population behaviour:

Force-based dynamics models. This model predicts the 3D movement of a single cell in the matrix, regulated by traction forces at both the front and rear end of the cell, forces due to cell protrusion into the matrix and viscous drag forces due to cell motility in the viscoelastic ECM (Zaman et al, 2005). This model present several important limitations: it is only valid for a single cell, while in vivo conditions normally include a population of cells; the change in shape of cells is not considered; mechanosensing mechanisms are considered phenomenologically; matrix remodelling due to cell migration is neglected. In fact, a more recent approach have been proposed by Borau et al (2011) in order to incorporate mechanosensing mechanisms to regulate cell migration obtaining results according to different experiments.

Multicellular spheroid migration. This model has been used to study the movement of cancer cell spheroids (McElwain and Petter, 1993). It is based on motion due to random walk, pressure gradients and chemotactic activity of cell aggregates. The main limitations are that

they do not consider important effects like matrix mechanical conditions.

Monte Carlo models. Zaman (2007) has also used Monte Carlo type models, using square lattices in 3D environment. The main drawback of this model is that it is not able to consider in a quantitative way important effects, like mechanical properties of the matrix, cell-matrix interaction and cell polarity.

4. Conclusions

In this chapter we have presented a number of key aspects of cell mechanics and mechanobiology. It is clear that the cell is a highly complex mechanical structure, in which mechanical and mechano-chemical events take place that have their own spatio-temporal dynamics. Clearly, the cell is not a uniformly stressed, passive and static continuous body. Instead, it is an active, highly dynamic mechanical structure, in which intracellular stresses will be heterogeneous and focused on specific cellular components, such as focal adhesion complexes and parts of the cytoskeleton. The fact that this structure is prestressed seems to be important for the cell to allow mechanical signals to be transduced from the extracellular environment up to the cell nucleus. The cell exhibits highly complex, non-linear and time dependent mechanical behaviour, requiring specific approaches for measuring and modelling these properties.

In order to explain the cell's ability to convert mechanical signals into a biochemical responses we have focused on the importance of focal adhesion complexes as primary centers for mechanosensing and mechanotransduction. Mechanical signals may be transduced into a biochemical response by mechanically-driven conformational changes of mechanosensory proteins, such as integrins. Integrins provide the cell with the ability to adhere to the extracellular matrix, while their cytoplasmic tail will bind to actin-binding proteins, in this way forming an essential part of a molecular mechanical network that bridges the extracellular and intracellular space. Due to this interconnected network, mechanochemical conversion in response to external (e.g. related to physiological activity, leading to ECM stresses and deformations) or internal (i.e. cell-generated, contractile) stresses may actually make use of the same mechanisms and intracellular components (Chen, 2008). The fact that such a common mechanism may exist has formed the basis for the development of computational models of mechanosensing that are able to study the cell mechanical response to external loading as well as substrate stiffness (Moreo et al., 2008). While integrin-mediated mechanotransduction is undoubtedly of key importance for explaining many *in vivo* and *in vitro* observations of mechanobiological phenomena, it may not explain the effect of mechanical loading conditions that do not lead to stress focusing on focal adhesion complexes, such as the effect of hydrostatic pressure on chondrocytes. In addition, other cellular structures, such as the primary cilium, have been identified as mechanosensors as well (Hoey et al., 2012).

Many experimental studies have demonstrated crosstalk between mechanical signaling and the activation of major molecular signaling pathways and related signal transducing molecules, like G proteins, MAPKs and RTKs. SFKs and Rho-GTPases can provide an important mechanism for crosstalk between integrin and growth factor activation (Huveneers and Danen, 2009). One of the major challenges in mechanobiology lies in the further unraveling of these crosstalk mechanisms, to identify molecules that are at the heart of mechanochemical conversion and their downstream targets, in this way establishing mechanochemical transduction pathways. Another major challenge is the integration of this molecular information in computational models of cell mechanobiology. Having a cell

computational model that can capture the spatio-temporal dynamics of and mutual feedback between mechanical and chemical signals and responses will lead to new insights in mechanobiological processes. Such a cell model could then be coupled to a mechanical model of the ECM, ultimately leading to multiscale models that enable to address the interplay between different time and length scales. Again, the development and validation of such models will be a major challenge for the future. Given the importance of mechanobiology for many physiological and pathophysiological processes, such models can increase our understanding on the way the mechanical microenvironment regulates cell fate.

Acknowledgements

The research work of JM García-Aznar was supported by the Spanish Ministry of Science and Innovation (DPI 2009-14115-CO3-01) (project part financed by the European Union, European Regional Development Fund).

Glossary

Adenosine triphosphate (ATP): a small molecules that is central to the generation of intracellular energy through its hydrolysis into adenosine diphosphate (ADP) or adenosine monophosphate (AMP)

Apoptosis: programmed cell death

Cell (or plasma) membrane: the layer that separates the cell interior from the extracellular environment. It is formed by a double layer of phospholipid molecules in which proteins are embedded, which e.g. function as channels to regulate molecular transport between the intracellular and extracellular space (such as ion channels) or receptors for extracellular signals (that can be soluble or matrix-bound).

Cell differentiation: the process by which a less specialised cell becomes a more specialised cell type, which is among others crucial for the development of multicellular organisms, or the establishment of tissue engineering strategies. The latter often make use of undifferentiated (or less differentiated) stem cells that need to be differentiated to mature, tissue-specific cell types.

Cell migration: a cell's ability to move, which may involve random motion as well as directed motion. At a more phenomenological level directionality depends on gradients in the concentration of extracellular matrix molecules (called haptotaxis), soluble factors (chemoattractants or repellants, called chemotaxis), stiffness of the extracellular matrix (called durotaxis) or mechanical stresses (called mechanotaxis).

Cell proliferation: a cell's ability to divide, leading to the growth of a cell population.

Cell signaling: a cell's ability to perceive microenvironmental signals and to respond to them, which relies on cascades of molecular interactions (called pathways) that are interrelated (called crosstalk) and that together form highly complex molecular systems (networks). Cell signaling governs basic cellular activities, such as migration, proliferation and differentiation, and forms the basis for development, growth, remodelling, regeneration and disease. A signaling pathway starts with an extracellular signal (called 'first messenger') that binds to a cell surface receptor, which, upon binding, will lead to alterations of intracellular molecules (called 'second messengers'). The binding of a receptor to an extracellular signal can lead to many responses, which ultimately regulate gene expression. Mechanotransduction can be seen as a specific case of signal transduction, where mechanical signals are responsible for or are mediating a certain biochemical response (which is likely to involve similar molecular players).

Collagen: a family of extracellular matrix proteins

Cytoplasm: the cell's interior, comprising the cytosol and all organelles, including the nucleus

Cytoskeleton: the cellular scaffold or skeleton within the cell's interior that controls the cell's structure and shape. Three type of cytoskeletal filaments can be discerned: actin filaments (or microfilaments), intermediate filaments and microtubules

Cytosol: an aqueous solution, containing a very complex mixture of molecules, such as proteins and filling the compartments of the cell's cytoplasm

Deoxyribonucleic acid (DNA): a molecule that encodes the genetic information of all living organisms. Specific sequences of pieces of DNA are referred to as genes.

Enzyme: a molecule (mostly a protein) that catalyses a certain metabolic reaction. Examples are kinases and phosphatases that catalyse phosphorylation and dephosphorylation reactions respectively.

Gene expression: the process in which the information encoded by a gene is converted into a gene product, which is often a protein. It involves the transcription (copying) of the gene into a molecule called messenger

RNA (mRNA, ribonucleic acid), which in turn is translated into a protein. The control of gene expression is fundamental to the control of a cell's structure and function, and is therefore also central to the control of cell differentiation and an organism's phenotype.

Growth factor: a molecule (often a protein) that stimulates cellular growth, proliferation and/or differentiation. It acts as an extracellular signaling molecule ('first messenger') that binds to a specific receptor on a target cell, and as such serves as a ligand for its receptor.

Homeostasis: a biological system's property to aim at maintaining certain microenvironmental variables at a constant level.

Mechanical signal: any mechanical variable that a tissue, cell or molecule is exposed to, such as a stress or strain. A mechanical signal can be imposed through an external load – in which case in this chapter we refer to it as 'mechanical stimulation' – or it can be generated by the cell itself. The word 'signal' also refers to its mediating role for cell signaling (see 'cell signaling').

Mechanosensing: a cell's ability to sense mechanical signals.

Mechanotransduction: a cell's ability to sense mechanical signals and convert them into a biochemical response.

Mitogen: a molecule (often a protein) that stimulates cell division (mitosis)

Molecular motor: a molecule (like a protein) that is able to convert chemical energy (which is e.g. obtained from the hydrolysis of ATP) into mechanical work (motion). A well-known example is myosin, which is responsible for muscle contraction, as well as for cellular contractility, through its interaction with actin. It obtains its energy through the hydrolysis of ATP.

Nucleus: a membrane-bound organelle (i.e. a subunit within a cell) found in eukaryotic cells that among others contains the genetic material (DNA)

Pathology: the study of disease

Persistence length: a property of a polymer chain that provides a statistical measure of the length of the chain along which it is persisting in a certain direction. The persistence length is proportional to the chain's bending stiffness.

Phosphorylation: the addition of a phosphate group to a molecule, such as a protein. Phosphorylation (and dephosphorylation, which is the opposite) of a molecule regulates its conformation and therefore activity.

Remodelling: dynamic process that leads to structural and/or compositional changes of biological matter in response to environmental signals. Depending on what is the subject of the remodeling process, we can e.g. discern cytoskeletal remodelling or tissue remodelling.

Stem cell: a cell that has the ability to self-renew (without losing its undifferentiated state) and that has the potential to differentiate into multiple specialized cell types. Examples are mesenchymal stem cells that can differentiate into different connective tissue cells, such as fibroblasts (fibrous tissue), chondrocytes (cartilage), osteoblasts (bone) and myoblasts (muscle).

Tensegrity: 'tensional integrity', a structural network that self-stabilises through internal tensional stress, which is counteracted by compression struts. In the context of cytoskeletal mechanics, microfilaments can be considered as tensional elements, while microtubules can act as compression struts.

Bibliography

- Adams M.A. and Dolan P. (2005). Spine biomechanics. *J Biomech.* **38**(10), 1972-1983.
- Amor N, Geris L, Vander Sloten J, Van Oosterwyck H (2009). Modelling the early phases of bone regeneration around an endosseous oral implant. *Comput Methods Biomech Biomed Engin.* **12**(4):459-68.
- An, S., Fabry, B., Mellema, M., Bursac, P., Gerthoffer, W., Kayali, U., Gaestel, M., Shore, S., and Fredberg, J. (2004). Role of heat shock protein 27 in cytoskeletal remodelling of the airway smooth muscle cell. *J. Appl. Physiol.*, **96**(5):1701-1713.
- Ando J. and Yamamoto K. (2009). Vascular mechanobiology: endothelial cell responses to fluid shear stress. *Circ. J* **73**(11), 1983-1992.
- Arruda E.M. and Boyce M.C. (1993). A three-dimensional constitutive model for the large stretch behavior of rubber elastic materials. *Journal of the Mechanics and Physics of Solids* **41**(2), 389-412.
- Bausch, A., Ziemann, F., Boulbitch, A., Jacobson, K., and Sackmann, E. (1998). Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry. *Biophys. J.*, **75**(4):2038-2049.
- Benhardt H.A. and Cosgriff-Hernandez E.M. (2009). The Role of Mechanical Loading in Ligament Tissue Engineering. *Tissue Engineering Part B: Reviews* **15**(4), 467-475.
- Borau C, Kamm RD, Garcia-Aznar JM (2011). Mechano-sensing and cell migration: a 3D model approach. *Phys Biol.* 2011 Dec; **8**(6):066008.
- Borau C, Kim T, Bidone T, García-Aznar JM, Kamm RD (2012). Dynamic mechanisms of cell rigidity sensing: insights from a computational model of actomyosin networks. *PLoS One.* 2012; **7**(11):e49174.
- Bromann P.A., Korkaya H., and Courtneidge S.A. (2004). The interplay between Src family kinases and receptor tyrosine kinases. *Oncogene* **23**(48), 7957-7968.
- Burridge K. and Wennerberg K. (2004). Rho and Rac take center stage. *Cell* **116**(2), 167-179.
- Bursac, P., Lenormand, G., Fabry, B., Oliver, M., Weitz, D., Viasnoff, V., Butler, J., and Fredberg, J. (2005). Cytoskeletal remodelling and slow dynamics in the living cell. *Nat. Mater.*, **4**(7):557-561.
- Butler D.L., Goldstein S.A., and Guilak F. (2000). Functional tissue engineering: the role of biomechanics. *J. Biomech. Eng* **122**(6), 570-575.
- Butler, J., Tolic-Nrrelykke, I., Fabry, B., Fredberg, J., (2002). Traction fields, moments, and strain energy that cells exert on their surroundings. *Am J Physiol Cell Physiol* **282**, C595-C605.

- Butler D.L., Goldstein S.A., Guldberg R.E., Guo X.E., Kamm R., Laurencin C.T., McIntire L.V., Mow V.C., Nerem R.M., Sah R.L., Soslowsky L.J., Spilker R.L., and Tranquillo R.T. (2009). The Impact of Biomechanics in Tissue Engineering and Regenerative Medicine. *Tissue Engineering Part B: Reviews* **15**(4), 477-484.
- Carter D.R., Beaupre G.S., Giori N.J., and Helms J.A. (1998). Mechanobiology of skeletal regeneration. *Clin. Orthop. Relat Res* (355 Suppl), S41-S55.
- Chang L. and Karin M. (2001). Mammalian MAP kinase signalling cascades. *Nature* **410**(6824), 37-40.
- Chen C.S. (2008). Mechanotransduction - a field pulling together? *Journal of Cell Science* **121**(20), 3285-3292.
- Clark C.B., McKnight N.L., and Frangos J.A. (2002). Strain and strain rate activation of G proteins in human endothelial cells. *Biochem. Biophys. Res. Commun.* **299**(2), 258-262.
- Darling E.M. and Athanasios K.A. (2003). Articular cartilage bioreactors and bioprocesses. *Tissue Eng* **9**(1), 9-26.
- Eliceiri B.P. (2001). Integrin and growth factor receptor crosstalk. *Circ. Res.* **89**(12), 1104-1110.
- Engler A.J., Sen S., Sweeney H.L., and Discher D.E. (2006). Matrix elasticity directs stem cell lineage specification. *Cell* **126**(4), 677-689.
- Gardel M.L., Shin J.H., MacKintosh F.C., Mahadevan L., Matsudaira P., and Weitz D.A. (2004). Elastic Behavior of Cross-Linked and Bundled Actin Networks. *Science* **304**(5675), 1301-1305.
- Georges P.C. and Janmey P.A. (2005). Cell type-specific response to growth on soft materials. *Journal of Applied Physiology* **98**(4), 1547-1553.
- Gerace L. and Huber MD (2012). I. Nuclear lamina at the crossroads of the cytoplasm and nucleus. *J Struct Biol.* **177**(1):24-31.
- Geris L, Schugart R, Van Oosterwyck H (2010). In silico design of treatment strategies in wound healing and bone fracture healing. *Philos Transact A Math Phys Eng Sci.* **368**(1920):2683-706.
- Geris L, Vander Sloten J, Van Oosterwyck H (2009). In silico biology of bone modelling and remodelling: regeneration. *Philos Transact A Math Phys Eng Sci.* **367**(1895):2031-53.
- Gilbert P.M., Havenstrite K.L., Magnusson K.E., Sacco A., Leonardi N.A., Kraft P., Nguyen N.K., Thrun S., Lutolf M.P., and Blau H.M. (2010). Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science* **329**(5995), 1078-1081.
- Gudi S.R., Clark C.B., and Frangos J.A. (1996). Fluid flow rapidly activates G proteins in human endothelial cells. Involvement of G proteins in mechanotransduction. *Circ. Res.* **79**(4), 834-839.
- Gudi S.R., Lee A.A., Clark C.B., and Frangos J.A. (1998). Equibiaxial strain and strain rate stimulate early activation of G proteins in cardiac fibroblasts. *Am. J. Physiol* **274**(5 Pt 1), C1424-C1428.
- Guilak F., Cohen D.M., Estes B.T., Gimble J.M., Liedtke W., and Chen C.S. (2009). Control of Stem Cell Fate by Physical Interactions with the Extracellular Matrix. *Cell Stem Cell* **5**(1), 17-26.
- Hamill O.P. and Martinac B. (2001). Molecular basis of mechanotransduction in living cells. *Physiol Rev* **81**(2), 685-740.
- Hansen C.A., Schroering A.G., Carey D.J., and Robishaw J.D. (1994). Localization of a heterotrimeric G protein gamma subunit to focal adhesions and associated stress fibers. *J. Cell Biol* **126**(3), 811-819.
- Hoey D.A., Downs M.E., and Jacobs C.R. (2012). The mechanics of the primary cilium: an intricate structure with complex function. *J. Biomech.* **45**(1), 17-26.
- Hu S., Chen J., Fabry B., Numaguchi Y., Gouldstone A., Ingber D.E., Fredberg J.J., Butler J.P., and Wang N. (2003). Intracellular stress tomography reveals stress focusing and structural anisotropy in cytoskeleton of living cells. *Am. J. Physiol Cell Physiol* **285**(5), C1082-C1090.
- Huebsch N., Arany P.R., Mao A.S., Shvartsman D., Ali O.A., Bencherif S.A., Rivera-Feliciano J., and Mooney D.J. (2010). Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nat. Mater.* **9**(6), 518-526.
- Humphrey J.D. (2008). Vascular adaptation and mechanical homeostasis at tissue, cellular, and sub-cellular levels. *Cell Biochem. Biophys.* **50**(2), 53-78.
- Huveneers S. and Danen E.H. (2009). Adhesion signaling - crosstalk between integrins, Src and Rho. *J. Cell Sci.* **122**(Pt 8), 1059-1069.
- Ingber D.E. (1997). Tensegrity: the architectural basis of cellular mechanotransduction. *Annual Review of Physiology* **59**(1), 575-599.
- Ingber D.E. (2002). Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ. Res.* **91**(10), 877-887.
- Ingber, D. (2003). Tensegrity I. Cell structure and hierarchical systems biology. *J.Cell.Sci.* **116**(7):1157-1173.
- Ingber D.E. (2003). Mechanobiology and diseases of mechanotransduction. *Ann. Med.* **35**(8), 564-577.
- Ingber D.E. (2006). Cellular mechanotransduction: putting all the pieces together again. *The FASEB Journal* **20**(7), 811-827.
- Ingber D.E. (2008). Tensegrity-based mechanosensing from macro to micro. *Progress in Biophysics and Molecular Biology* **97**(2-3), 163-179.
- Iwasaki H., Eguchi S., Ueno H., Marumo F., and Hirata Y. (2000). Mechanical stretch stimulates growth of vascular smooth muscle cells via epidermal growth factor receptor. *Am. J. Physiol Heart Circ. Physiol* **278**(2), H521-H529.
- Jamali Y, Azimi M, Mofrad MR (2010). A sub-cellular viscoelastic model for cell population mechanics. *PLoS One.* **5**(8). pii: e12097.
- Janmey P.A. and Weitz D.A. (2004). Dealing with mechanics: mechanisms of force transduction in cells. *Trends in Biochemical Sciences* **29**(7), 364-370.
- Janmey PA, McCormick ME, Rammensee S, Leight JL, Georges PC, MacKintosh FC (2007). Negative normal stress in semiflexible biopolymer gels. *Nat Mater.* **6**(1):48-51.
- Javierre E., Moreo P., Doblaré M, García-Aznar, JM (2009). Numerical modelling of a mechano-chemical theory for wound contraction analysis. *International Journal of Solids and Structures*, **46**: 3597-3606.
- Kim T., Hwang W., Lee H., and Kamm R.D. (2009). Computational Analysis of Viscoelastic Properties of Crosslinked Actin Networks. *PLoS Comput Biol* **5**(7), e1000439.
- Kollmannsberger P, Fabry B (2011). Linear and non-linear rheology of living cells. *Annu Rev Mater Res* **41**:75-97.
- Kumar S. and Weaver V. (2009). Mechanics, malignancy, and metastasis: The force journey of a tumor cell. *Cancer and Metastasis Reviews* **28**(1), 113-127.
- Legant, W., J.S.Miller, Blakely, B., Cohen, D., Genin, G., Chen, C., (2010). Measurement of mechanical traction exerted by cells within three dimensional matrices. *Nat Methods* **7**(12), 969-71.
- Lenormand, G., Chopin, J., Bursac, P., Fredberg, J., and Butler, J. (2007). Directional memory and caged dynamics in cytoskeletal remodelling. *Biochem.Biophys.Res.Comm.*, **360**(4):797-801.
- Lutolf M.P., Gilbert P.M., and Blau H.M. (2009). Designing materials to direct stem-cell fate. *Nature* **462**(7272), 433-441.
- MacKintosh F.C., Kas J., and Janmey P.A. (1995). Elasticity of semiflexible biopolymer networks. *Physical Review Letters* **75**(24), 4425-4428.
- Masuda H., Zhuang Y.J., Singh T.M., Kawamura K., Murakami M., Zarins C.K., and Glagov S. (1999). Adaptive remodelling of internal elastic lamina and endothelial lining during flow-induced arterial enlargement. *Arterioscler. Thromb. Vasc. Biol* **19**(10), 2298-2307.
- Meyer C.J., Alenghat F.J., Rim P., Fong J.H., Fabry B., and Ingber D.E. (2000). Mechanical control of cyclic AMP signalling and gene transcription through integrins. *Nat Cell Biol* **2**(9), 666-668.

- Mitra S.K. and Schlaepfer D.D. (2006). Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr. Opin. Cell Biol* **18**(5), 516-523.
- Mofrad MRK and Kamm RD Eds (2006). *Cytoskeletal Mechanics: Models and Measurements*, Cambridge University Press.
- Mofrad MRK and Kamm RD Eds (2009). *Cellular Mechanotransduction: Diverse Perspectives From Molecules to Tissues*, Cambridge University Press.
- Moreo P., Garcia-Aznar J.M., and Doblare M. (2008). Modelling mechanosensing and its effect on the migration and proliferation of adherent cells. *Acta Biomater.* **4**(3), 613-621.
- Moreo P, Gaffney EA, Garcia-Aznar JM, Doblare M (2010). On the modelling of biological patterns with mechanochemical models: Insights from analysis and computation. *Bull Math Biol.* **72**(2):400-31.
- Moro L., Dolce L., Cabodi S., Bergatto E., Boeri E.E., Smeriglio M., Turco E., Retta S.F., Giuffrida M.G., Venturino M., Godovac-Zimmermann J., Conti A., Schaefer E., Beguinot L., Tacchetti C., Gaggini P., Silengo L., Tarone G., and Defilippi P. (2002). Integrin-induced epidermal growth factor (EGF) receptor activation requires c-Src and p130Cas and leads to phosphorylation of specific EGF receptor tyrosines. *J. Biol Chem.* **277**(11), 9405-9414.
- Murray JD. On the mechanochemical theory of biological pattern formation with application to vasculogenesis. *C R Biol.* 2003 Feb;326(2):239-52.
- Na S., Collin O., Chowdhury F., Tay B., Ouyang M., Wang Y., and Wang N. (2008). Rapid signal transduction in living cells is a unique feature of mechanotransduction. *Proc. Natl. Acad. Sci. U. S. A* **105**(18), 6626-6631.
- Nerem R.M. (2003). Role of mechanics in vascular tissue engineering. *Biorheology* **40**(1-3), 281-287.
- Neves S.R., Ram P.T., and Iyengar R. (2002). G Protein Pathways. *Science* **296**(5573), 1636-1639.
- Oppermann H., Levinson A.D., Varnus H.E., Levintow L., and Bishop J.M. (1979). Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene (src). *Proc. Natl. Acad. Sci. U. S. A* **76**(4), 1804-1808.
- Or-Tzadikario S, Gefen A. Confocal-based cell-specific finite element modeling extended to study variable cell shapes and intracellular structures: the example of the adipocyte. *J Biomech* **44**(3):567-73.
- Orr A.W., Helmke B.P., Blackman B.R., and Schwartz M.A. (2006). Mechanisms of mechanotransduction. *Dev. Cell* **10**(1), 11-20.
- Palmer J.S. and Boyce M.C. (2008). Constitutive modelling of the stress-strain behavior of F-actin filament networks. *Acta Biomater.* **4**(3), 597-612.
- Peeters EA, Oomens CW, Bouten CV, Bader DL, Baaijens FP (2005). Viscoelastic properties of single attached cells under compression. *J Biomech Eng.* **127**(2):237-43.
- Plopper G.E., McNamee H.P., Dike L.E., Bojanowski K., and Ingber D.E. (1995). Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex. *Mol Biol Cell* **6**(10), 1349-1365.
- Prokharau PA, Vermolen FJ, García-Aznar JM (2012). Model for direct bone apposition on pre-existing surfaces, during peri-implant osseointegration. *J Theor Biol.* **304**:131-42.
- Puig-De-Morales M, Grabulosa M, Alcaraz J, Mullol J, Maksym GN, Fredberg JJ, Navajas D (2001). Measurement of cell microrheology by magnetic twisting cytometry with frequency domain demodulation. *J Appl Physiol.* **91**(3):1152-9.
- Rey R, Garcia-Aznar JM. (2013). A phenomenological approach to modelling collective cell movement in 2D. *Biomech Model Mechanobiol.* (on-line).
- Rossomando A.J., Payne D.M., Weber M.J., and Sturgill T.W. (1989). Evidence that pp42, a major tyrosine kinase target protein, is a mitogen-activated serine/threonine protein kinase. *Proc. Natl. Acad. Sci. U. S. A* **86**(18), 6940-6943.
- Sabass, B., Gardel, M., Waterman, C., Schwarz, U., 2008. High resolution traction force microscopy based on experimental and computational advances. *Biophysical Journal* **94**, 207–220.
- Slager C.J., Wentzel J.J., Gijzen F.J.H., Thury A., van der Wal A.C., Schaar J.A., and Serruys P.W. (2005). The role of shear stress in the destabilization of vulnerable plaques and related therapeutic implications. *Nat Clin Pract Cardiovasc Med* **2**(9), 456-464.
- Slomka N, Gefen A. Confocal microscopy-based three-dimensional cell-specific modeling for large deformation analyses in cellular mechanics. *J Biomech.* **43**(9):1806-16.
- Soung Y.H., Clifford J.L., and Chung J. (2010). Crosstalk between integrin and receptor tyrosine kinase signaling in breast carcinoma progression. *BMB. Rep.* **43**(5), 311-318.
- Stehelin D., Fujita D.J., Padgett T., Varnus H.E., and Bishop J.M. (1977). Detection and enumeration of transformation-defective strains of avian sarcoma virus with molecular hybridization. *Virology* **76**(2), 675-684.
- Sunyer R, Trepat X, Fredberg JJ, Farré R, Navajas D (2009). The temperature dependence of cell mechanics measured by atomic force microscopy. *Phys Biol.* **6**(2):025009.
- Turner C.H. (1998). Three rules for bone adaptation to mechanical stimuli. *Bone* **23**(5), 399-407.
- Valero C., Javierre E, García-Aznar JM., Gómez-Benito MJ. (2012). Numerical modelling of the angiogenesis process in wound contraction, *Biomechanics and Modelling in Mechanobiology*, DOI: 10.1007/s10237-012-0403-x.
- van der Meulen M.C.H. and Huiskes R. (2002). Why mechanobiology?: A survey article. *Journal of Biomechanics* **35**(4), 401-414.
- Vermolen FJ, Gefen A (2012a). A semi-stochastic cell-based formalism to model the dynamics of migration of cells in colonies. *Biomech Model Mechanobiol.* **11**(1-2):183-95.
- Vermolen FJ, Gefen A (2012b). A phenomenological model for chemico-mechanically induced cell shape changes during migration and cell-cell contacts. *Biomech Model Mechanobiol.* (on-line).
- Vermolen FJ, Gefen A (2013). A semi-stochastic cell-based model for in vitro infected 'wound' healing through motility reduction: a simulation study. *J Theor Biol.* (on-line).
- Versaevol M, Grevesse T, Gabriele S (2012). Spatial coordination between cell and nuclear shape within micropatterned endothelial cells. *Nat Commun.* **3**:671. doi: 10.1038/ncomms1668.
- Wang J. and Thampatty B. (2006). An Introductory Review of Cell Mechanobiology. *Biomechanics and Modelling in Mechanobiology* **5**(1), 1-16.
- Wang N., Butler J.P., and Ingber D.E. (1993). Mechanotransduction across the cell surface and through the cytoskeleton. *Science* **260**(5111), 1124-1127.
- Wang N., Tytell J.D., and Ingber D.E. (2009). Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. *Nat Rev Mol Cell Biol* **10**(1), 75-82.
- Wood ST, Dean BC, Dean D (2012). A Computational Approach to Understand Phenotypic Structure and Constitutive Mechanics Relationships of Single Cells. *Ann Biomed Eng.* (on-line).
- Yoshida M., Westlin W.F., Wang N., Ingber D.E., Rosenzweig A., Resnick N., and Gimbrone M.A., Jr. (1996). Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton. *J. Cell Biol* **133**(2), 445-455.
- Zajac A.L. and Discher D.E. (2008). Cell differentiation through tissue elasticity-coupled, myosin-driven remodelling. *Curr. Opin. Cell Biol.* **20**(6), 609-615.
- Zwick E., Bange J., and Ullrich A. (2001). Receptor tyrosine kinase signalling as a target for cancer intervention strategies. *Endocrine-Related Cancer* **8**(3), 161-173.

Biographical Sketches

Hans Van Oosterwyck (DOB 02.02.1972) holds an MSc degree in Materials Engineering (1995) and a PhD degree in Engineering (2000), both obtained at KU Leuven (Leuven, Belgium). He has been a postdoctoral fellow at the AO Research Institute (Davos, Switzerland) in 2004-2005 and a visiting scientist at the University of Zaragoza (Spain) in 2009.

He currently is a professor at the Biomechanics section (Mechanical Engineering Department) at KU Leuven, where he is heading the mechanobiology and tissue engineering research group, and a visiting professor at Ghent University (Ghent, Belgium). He is a member of Prometheus, the Leuven R&D Division for Skeletal Tissue Engineering. He was recently (2012) awarded an ERC Starting Grant on the role of cell-matrix interaction in angiogenesis. He is (co-)author of 63 ISI indexed journal papers and has an h-index of 18. His research focuses on the development of quantitative tools for unraveling the role of the microenvironment for cell fate, in particular the development of multiscale computational models for studying the importance of mechanics and mass transport for bone regeneration and angiogenesis.

Prof. Van Oosterwyck is a member of the European Society of Biomechanics (ESB) and has been an ESB Council Member since 2006. Currently he is the President of the ESB (2012-2014).

Liesbet Geris (DOB 04.06.1979) received her MSc degree in Mechanical Engineering in 2002 from the Katholieke Universiteit Leuven (Leuven, Belgium) and her PhD degree in Engineering in 2007 from the KU Leuven (Leuven, Belgium), both summa cum laude. In 2007 she worked for 6 months as an academic visitor at the Centre of Mathematical Biology of Oxford University.

She currently is an assistant professor in Biomechanics at the Department of Aerospace and Mechanical Engineering at the university of Liège and guest professor at the Department of Mechanical Engineering of the KU Leuven, Belgium. She is scientific coordinator of the Leuven R&D division for skeletal tissue engineering, Prometheus (9 PIs, 12 postdocs, 20 PhD students) and is co-PI of a large number of interdisciplinary projects in the field of (bone) tissue engineering (funded through university, government and industry). She was recently (2011) awarded an ERC Starting Grant. She is the author of 40 ISI indexed journal papers (h-index 11), 5 book chapters, 32 full conference proceedings and 41 conference abstracts. She is the editor of a book on Computational modelling in Tissue Engineering (Berlin-Heidelberg, Germany: Springer-Verlag, 2012). Her research interests encompass the mathematical modelling of bone regeneration during fracture healing, implant osseointegration and tissue engineering applications. The phenomena described in the models reach from the tissue level, over the cell level, down to the molecular level.

Prof. Geris is member of the European society of Biomechanics and the European Society for Mathematical and Theoretical Biology. She has received a number of awards, including the Student Award of the European Society of Biomechanics (ESB, 2006), the Young Investigator Award of the International Federation for Medical and Biological Engineering (IFMBE, 2008) and the Taylor & Francis award for outstanding innovation in computational methods in biomechanics and biomedical engineering (2010).

José Manuel García Aznar (DOB 04.01.1970) received his BSc in Mechanical Engineering in 1995 from the Universidad de Zaragoza (Zaragoza, Spain) and his PhD degree in Computational Mechanics in 1999 from the same university, both summa cum laude. In 2001, he worked for 6 months as a postdoctoral researcher at the Center for Science and Technology in Medicine (University of Keele, United Kingdom), working on computational simulation of tissue differentiation in bone fracture healing.

He currently is Full Professor in Structural Mechanics at the Department of Mechanical Engineering at the Universidad de Zaragoza. He was recently (2012) awarded an ERC Starting Grant. He is the author of 76 ISI indexed journal papers (h-index 15), 20 book chapters and 80 contributions to international conferences. His research interests focus on the mathematical modelling of mechanics of hard tissues, mechanobiology of skeletal tissue regeneration and tissue engineering, tissue growth and development and cell mechanics.

Prof. García-Aznar is member of the European Society of Biomechanics (ESB) and has been a Council Member (2004-2012) and Vice-President (2008-2012) of the ESB.